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FULL PAPER

Antioxidant activity and sensory evaluation of a cold dairy-based beverage enriched with *Sargassum polycystum* C. Agardh extract in sunflower oil

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Abstract

Natural based functional food and beverages have gained market interest in the last decade. Functional beverages, especially those enriched with nutrients and bioactive compounds, are of particular interest. Maintaining the nutritional or bioactive activity of these components along with their sensory attributes in the final product remains to be a challenge. Health benefits from bioactive substances, like fucoxanthin and phlorotannins, in marine algae makes it a promising commodity of an archipelago country. However, nutritional components, their stability, and an understanding of their nutritional and bioactive component availability require further investigation. Here we prospected natural and sustainable antioxidant additives from brown algae, *Sargassum polycystum* C Agardh, to enrich a cold dairy-based beverage. Food-grade extraction of *S. polycystum* was done by using 96% Ethanol to macerate dried powder algal. Identification of fucoxanthin in the extract was performed using High-Performance Liquid Chromatography (HPLC) (Prominance Shimadzu). We employed the 2,2-diphenyl-1-picrylhydrazyl method to quantify the antioxidant activity of extract alone and extract in three beverage formulations containing 0.02, 0.05, and 0.1 mg/cup *S. polycystum* extract. Sensory evaluation and viscosity analysis comparing our product to the commercially available product was also performed. Results showed that the combination of *S. polycystum* extract with sunflower oil was able to maintain antioxidant activity when stored at 4°C. Formulation 2 of dairy-based beverages passed the sensory evaluation. This study was the first to report the application of *S. polycystum* extract on a cold dairy-based beverage. This product was developed considering the low stability of the bioactive compound, fucoxanthin. More comprehensive sensory evaluation and optimization for large scale production are highly required.

1. Introduction

In the past decade, there has been growing interest in the consumption of natural based-functional foods as they can potentially offer health benefits, such as reducing the risk of chronic diseases and improving the physiological conditions with almost no side effects (Ozen *et al*., 2012; Iwatani and Yamamoto, 2019; Jędrusek-Golińska *et al*., 2020). Functional food can be categorized into a whole, fortified, or enriched food and they can be consumed as part of the diet on a regular basis (Vicentini *et al*., 2016; Jędrusek-Golińska *et al*., 2020). The aim of this study was on the development of a functional beverage as it represents one of the largest and fastest-growing market sectors along with food and supplement. The global market of functional beverages has been increasing at a compound annual growth rate (CAGR) of 4.6% since 2015. Lockdowns and social distancing imposed in 2020 resulted in a 2.5% decrease, however, the market is expected to recover and grow at a CAGR of 8.07% between 2021 and 2023 (ResearchAndMarkets.com, 2020). The feasibility to incorporate nutrients and bioactive compounds result in high demand for functional beverages (Tolun and Altintas, 2019).

One of the fundamental concerns when developing functional products is whether to select the nutritional or bioactive component to maintain product stability. Desirable nutrients and bioactive compounds in

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functional beverages include antioxidants, dietary fibres, prebiotics, proteins, peptides, unsaturated fatty acids, minerals, and vitamins (Grumezescu and Holban, 2019). There are at least 8 categories of functional beverages such as dairy-based beverages, probiotic drinks, energy drinks, sports drinks, meal replacers, health and wellness, caffeinated beverages, weight management, vegetable, and fruit beverages (Tolun and Altintas, 2019). Indication of these functional beverages is either to improve the general and physical conditions or decrease the risk of the prognosis of diseases (Tolun and Altintas, 2019). Specific health benefits such as the reduced risk of cancer, improved immune system, improving physical and mental condition, anti-stress, anti -ageing, antioxidant, and anti-inflammatory properties help in the development of new products with suitable processing technologies (Shahidi and Alasalvar, 2016). Besides chemical and functional properties of the beverage, taste, texture, flavour, and appearance must also be taken into consideration to produce a safe, high quality, and market value product (Andersen *et al*., 2019; Wang, Mielby, Junge *et al*., 2019).

Marine algae, including brown algae, is a potential raw material providing health benefits from their bioactive substances (Afonso *et al*., 2019; Leandro *et al*., 2019). Notable bioactive constituents in brown alga are phlorotannins, polyphenol, phytosterol, as well as distinct marine carotenoids, namely fucoxanthin (Li *et al*., 2018; Hakim and Patel, 2020). Efforts made to increase the number of marine algal-based product includes investigation of the nutritional composition of algal species growing in different geographical regions and seasons, improving the stability of bioactive substances, understanding the bioavailability of nutritional and bioactive compounds and their effect on human metabolism and gut microbiome (Wu *et al*., 2014; Wells *et al*., 2017; Afonso *et al*., 2019; Mok *et al*., 2018; Zhao *et al*., 2019).

The aim of this study is to determine the stability of brown algae, *Sargassum polycystum* C. Agardh extracts from species growing in West Nusa Tenggara. Extract stability, based on the antioxidant activity, was improved by the addition of sunflower oil. The resulting stabilized extract was used to enrich milk resulting in a functional dairy-based beverage. Ethanolic extract of *S.polycystum* contains major bioactive substances contained in brown algae, including fucoxanthin (Li *et al*., 2018; Hakim and Patel, 2020; Lourenço-Lopes *et al*., 2020). The use of sunflower oil improves the solubility of many of the less polar bioactive substances contained in the extract (Peng *et al*., 2011; Hakim and Patel, 2020). In addition, sunflower oil is also high in oleic acid (up to ~73%) and low in stearic acid (~21% decrease), making this oil beneficial for human health (Garcés *et al*., 2009; Tupe *et al*., 2015; Anushree *et al*., 2017). Ultra-high-temperature (UHT) milk is considered microbially stable, has a relatively long shelf-life of 34–36 weeks when stored at temperatures between 4-20°C, and is available at affordable prices (Anema, 2019; Karlsson *et al*., 2019). Mok *et al*. (2018) suggest that fucoxanthin *in vitro* and *in vivo* studies show increased fucoxanthin bioavailability in fortified milk (Mok *et al*., 2018). This study developed functional beverages using cold treatment (4°C) to prevent degradation of bioactive compounds contained in *S. polycystum* that are sensitive to high temperature and acidic pH (Wu *et al*., 2014; Zhao *et al*., 2019). Antioxidant activity of the individual ingredients, as well as post-product formulation, was assessed and sensory evaluation was done.

2. Materials and methods

2.1 Materials

Sargassum polycystum was collected from Sumbawa district, West Nusa Tenggara in April – May 2019. Morphological identification was performed by a senior botanist and marine biologist at the Research Center for Oceanography, Indonesian Institute of Sciences. 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, DMSO were purchased from Sigma-Aldrich USA; Ethanol and distilled water were obtained as an analytical grade. All snack bar ingredients were purchased from a supermarket in South Jakarta.

2.2 Extraction

Sargassum polycystum was dried using an oven at 40°C for 48 hours. The whole dried algae were grounded using a food processor and the resulting powder was macerated in 96% Ethanol (5:1 v/v) for 48 hrs. The extract was collected and the remaining solvent was evaporated using a vacuum rotary evaporator at 40°C to obtain a solid-liquid extract. The extract was stored in sealed vials and kept at 4°C until further analysis. The extraction process was carried out in a low light environment and apparatus.

2.3 High-Performance Liquid Chromatography-PDA

Analysis of fucoxanthin content was performed using High-Performance Liquid Chromatography (HPLC) (Prominance Shimadzu) equipped with LC-20AD and SPD-M20A diode array. Solid-phase used was C30 YMC (150 mm × 4.6 mm ID. S-5 µm). Methanol, methyl tert-butyl ether (MTBE), and aquadest HPLC grade were used as mobile phase with flow rate was maintained at 1 mL/minute isocratic. The sample was filtered using 0.20 µm Minisart filters, then 20 µL was injected on the loop-injector. Detection of

fucoxanthin was performed using a photodiode array (190-800 nm) at 446 nm. Fucoxanthin standard (Sigma-Aldrich) was used as standard.

2.4 Antioxidant assay

Antioxidant activity of all ingredients was measured using the DPPH method as described in a previous study (Brand-Williams *et al*., 1995). In brief, samples were diluted in Ethanol 96% and diluted in the same solvent for the assay. A serially diluted sample or standard solution was mixed with 1 mL of 0.05 mM DPPH in Ethanol 96%. The mixture was incubated for 30 mins and in the dark. Absorbance (A1) was read at 517 nm. The absorbance of a blank (A0, ethanol instead of extract) was recorded at the same wavelength. Scavenging ability (%) was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [\frac{(A0-A1)}{A0}] \times 100$$

2.5 Beverage formulation

The different formulation used is mentioned in Table 1. Tween-80 was mixed gently with milk followed by stirring at 2000 rpm, 100°C for 15 mins. Carboxymethyl cellulose (CMC) was added gradually to prevent clumping. Subsequently, sugar was added to the mixture and stirred gently. The homogenized mixture was cooled to room temperature for about 1 hr. *Sargassum polycycstum* extract was fortified into milk and stirred at 2000 rpm at room temperature and in the absence of light for 30 mins. The beverage mixture was stored at 4°C in a dark and well-sealed container.

Table 1. Composition of the beverage

Ingredient	Formula 1	Formula 2	Formula 3
White milk full cream (mL/cup)	125	125	125
Tween-80 (g/cup)	0.1	0.1	0.1
CMC (g/cup)	0.2	0.2	0.2
Sunflower oil (g/cup)	1.5	1.5	1.5
Sugar (g/cup)	5	5	5
S. polycystum extract (g/cup)	0.02	0.05	0.1

The stability of *S. polycystum* extract in sunflower oil at 4°C was also measured based on their antioxidant activity. DPPH assay was performed as mentioned earlier. Extract to sunflower oil ratio follows Table 1. This test was done on day-0, 3, and 7.

2.6 Sensory test

An organoleptic test was performed by 35 untrained panellists between 16 and 60 years old. Parameters observed include taste, appearance, texture, aftertaste, and overall appearance. Each parameter was scored on a scale between 1 (least acceptable) to 9 (most acceptable) (Meilgaard MC, Civille GV, 2016).

2.7 Beverage viscosity

The viscosity of the most favoured formula was measured and compared to unfortified milk and commercially available dairy-based beverage. Analysis was performed using Brookfield LVT-230 viscometer. Five hundred microliter of each sample was placed in a container and placed under the machine rotor. Spindel number 1 at 6 rpm was used to measure viscosity (expressed as centipoise (cp)) after multiplying by 10.

2.8 Statistics

IBM SPSS Statistics 24 Commuter software was used and data was analyzed using one-way ANOVA followed by Duncan analysis to identify the presence of significant differences between the different formulations with a 95% confidence interval. All data were presented as average ± standard deviation.

3. Results and discussion

3.1 Sargassum polycystum extract and the presence of fucoxanthin

Ethanolic extract of *S. polycystum* was turquoise in colour with a paste-like consistency. Qualitative analysis of fucoxanthin was performed using an HPLC and its presence was detected at 448 nm with a retention time of 4.43 minutes (Figure 1). This is in accordance with results obtained from fucoxanthin standard where the peak was observed at a retention time of 4.329 mins and in a previous study *(Piovan et al*., 2013). The concentration of fucoxanthin obtained was 0.37 mg/g (Figure 1). *Sargassum polycystum* C. Agardh was first identified as promising brown algae growing in West Nusa Tenggara. This study reports the presence of an important carotenoid, fucoxanthin, at a comparable concentration to *Sargassum* species described by Xiao *et al*. (2012) (0.20 mg/g) and by Renhoran *et al*. (2017) (0.47 mg/g). Geographical differences affect the genetic profiles of marine algae, their defence mechanism

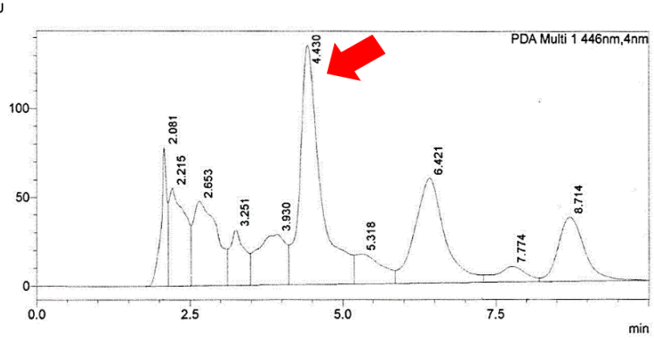

Figure 1. Chromatogram of *S. poycystum* extract and fucoxanthin was detected at Rt 4.43

towards their living environment and hence can affect the type and amount of bioactive components (Saleh and Elatroush, 2020). Industrial-scale extraction is required (Lourenço-Lopes *et al*., 2020).

3.2 Antioxidant activity of Sargassum polycystum extract, sunflower oil, and its combination in beverage formula

The ability to scavenge free radicals was used to assess antioxidant activity and is stated as the concentration of a test compound to inhibit 50% of free radical (IC_{50}). The lower IC_{50} means better antioxidant activity. Ascorbic acid was used as a positive control. As seen in Table 2, sunflower oil has the highest antioxidant activity when compared to *S. polycystum* extract.

Table 2. Antioxidant activity (IC_{50} value)

Ingredientt	IC_{50} (mg/mL)
Ascorbic acid	$18\times10^{-4}\pm0.01^{a}$
S. polycystum extract	0.16 ± 0.02^{b}
Sunflower oil	0.06 ± 0.08^{c}

Values are presented as mean±standard deviation. Values with different superscript are significantly different (P<0.05).

Antioxidant activity of the extract results from the presence of phlorotannin (Boi *et al*., 2016; Cuong *et al*., 2016) and fucoxanthin (Sachindra *et al*., 2007; Miyashita *et al*., 2020) - prominent antioxidants commonly found in *Sargassum* species. The phenolic pool in phlorotannin contributes to the hydrogen donating ability, exhibits high free radical scavenging activity, especially against $DPPH^{\bullet}$ and $O_2^{\bullet}$. Polyphenols with multiple hydroxyl groups also exhibit high redox potential, protecting from free radicals formed as by-products of mitochondrial metabolism (Mathew *et al*., 2015). Fucoxanthin, on the other hand, exhibits antioxidant activity due to the presence of conjugated bonds that are able to stabilize free radicals (Peng *et al*., 2011; Miyashita *et al*., 2020). Similar to the composition of olive oil, sunflower oil is rich in oleic acid and possesses a strong ability to scavenge free radicals, including DPPH (Xiang *et al*., 2017). Oleic acid also increases the Superoxide Dismutase (SOD) activity of cadmium-treated rat organs and reduces the level of superoxide radical O^{2-} (Wang, Zhang, Fang *et al*., 2019). Vitamin E, often contained in sunflower oil can synergistically increase antioxidant activity (Garcés *et al*., 2009; Aldini *et al*., 2010).

Antioxidant activity of *S. polycystum* in combination with sunflower oil in formulation most-favoured by the panellists, formula 2 was also measured (Figure 2). The ability of *S. polycystum* extract to maintain antioxidant activity was higher than unfortified extracts (control) and the activity was maintained up to day-7 (Figure 2).

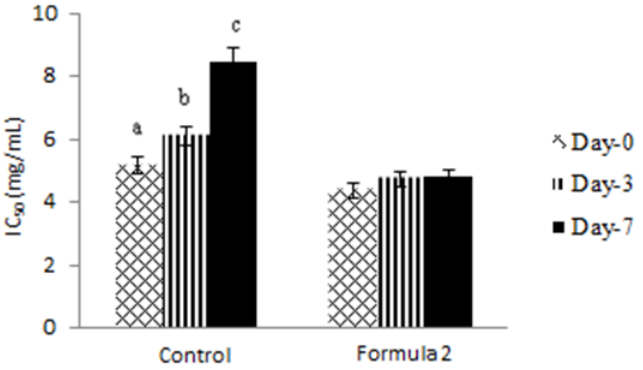

Figure 2. Antioxidant activity of formula 2 (with sunflower oil and extract) and control (without sunflower oil and extract). Bars with different notations are significantly different (P<0.05).

Combining *S. polycystum* extract with sunflower oil was able to maintain antioxidant activity. Further analysis is required to pinpoint the single bioactive compound contained in each ingredient that is responsible for this activity (Pérez-Gálvez *et al*., 2000; Sunil *et al*., 2015; Susanti *et al*., 2020). The formulation of beverage at 4°C maintained the stability of bioactive compounds present in the extract and prevented oxidation of sunflower oil (Guiotto *et al*., 2014). In addition, the presence of bioactive compounds in sunflower oil such as oleic acid and tocopherol may have also maintained antioxidant activity (Rao *et al*., 2007; Anushree *et al*., 2017; Wang *et al*., 2019).

3.3 Beverage formulation and sensory test

A combination of extract and sunflower oil was applied to develop a beverage with additional increased antioxidant activity. The panellists rated formula 2 as the best formula (Table 3). Although formula 3 contained the highest concentration of extract, it wasn't the most favoured amongst panellists. This can be attributed to the bitter taste that may have been caused by the *Sargassum* extract (Chengkui and Junfu, 1984; Liu *et al*., 2012; Encyclopedia, 2021). Tween 80 could have also contributed to the bitter taste (Rowe *et al*., 2009).

Table 3. Sensory analysis of formula 1, 2, and 3

Attribute	Formula 1	Formula 2	Formula 3
Color	5.60 ± 0.87^{a}	5.85 ± 1.25^{a}	5.75 ± 1.03^{a}
Aroma	4.60 ± 0.87^{a}	4.18 ± 0.81^{a}	4.40 ± 0.81^{a}
Taste	6.38 ± 1.20^{a}	6.27 ± 1.42^{a}	3.83 ± 1.01^{b}
Viscosity	5.28 ± 1.06^{a}	5.18 ± 1.11^{a}	5.15 ± 1.33^{a}
Aftertaste	5.28 ± 1.22^{a}	5.45 ± 1.50^{a}	3.33 ± 1.00^{b}
Overall	5.65 ± 0.85^{a}	6.5 ± 1.04^{b}	4.23 ± 0.93^{c}

Values are presented as mean±standard deviation. Values with different superscript within the same row are significantly different (P<0.05).

3.4 Viscosity test

Formula 2 has a viscosity of 135 cp, while the control formula that was not fortified with *Sargassum* has a viscosity value of 130 cp, and the commercial product was 359 cp. No significant difference was found between formula 2 and the control ($p>0.05$). The surfactant agent used was able to improve viscosity while still maintaining organoleptic scores (Figure 3). In this study, CMC was used as a thickening agent, surfactant enhancer, as well as a stabilizer. CMC has been widely used in the field of food production (Arancibia *et al*., 2013; Meng *et al*., 2018). The addition of CMC increases viscosity to desirable rheological and consistency (Arancibia *et al*., 2013).

4. Conclusion

Sargassum polycystum extract and sunflower oil are known to have individual antioxidant activity and are able to maintain this even when combined. This combination resulted in a dairy-based beverage with acceptable sensory attributes. This study presents the application of underexplored brown algae found in remote areas as a functional food that is cheap, safe, stable, and healthy. A study on product variation involving the greater scope of sensory evaluation and industrial scaling up is necessary.

Conflict of interest

The authors declare no conflict of interest.

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